

Molecular evidence for hybridization between invasive *Solidago canadensis* and native *S. virgaurea*

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Abstract Hybridization between alien and native species is biologically very important and could lead to genetic erosion of native taxa. *Solidago* × *niederederi* was discovered over a century ago in Austria and described by Khek as a natural hybrid between the alien (nowadays regarded also as invasive) *S. canadensis* and native *S. virgaurea*. Although interspecific hybridization in the genus *Solidago* is considered to be relatively common, hybrid nature of *S. × niederederi* has not been independently proven using molecular tools, to date. Because proper identification of the parentage for the hybrid *Solidago* individuals solely based on morphological features can be misleading, in this paper we report an additive polymorphism pattern expressed in the ITS sequences obtained from individuals representing *S. × niederederi*, and confirm the previous hypothesis that the parental species of this hybrid are *S. canadensis* and *S. virgaurea*. Additionally, based on variability at the cpDNA *rpl32-trnL* locus, we showed that in natural populations hybridization occurs in both directions.

Keywords Hybrid · Invasive species · ITS, *rpl32-trnL* · Sequencing · *Solidago* × *niederederi*

Introduction

Solidago canadensis L. (Asteraceae), a North American species, was introduced to Europe as an ornamental plant in the 17th century (Kowarik 2003), and in the 19th and 20th centuries became naturalized in many European countries (Weber 1997). Currently, it is regarded as an invasive species (Kabuce and Priede 2010). *Solidago virgaurea* L., in its narrow sense (*sensu stricto*), is a native European species (Slavík 2004; Kiełtyk and Mirek 2014). Spontaneous hybridization between the alien *S. canadensis* and native *S. virgaurea* in Europe was pointed out over a century ago, when Khek (1905) described the hybrid *S. × niederederi* Khek from Austria. Since then, *S. × niederederi* has been reported from several countries in Europe, including the United Kingdom, Sweden, Denmark, Norway, Germany, Poland, Lithuania and Russia (Nilsson 1976; Burton 1980; Sunding 1989; Mayorov et al. 2012; Pliszko 2013, 2015; Karpavičienė and Radušienė 2016).

Both parental species are morphologically very variable and their taxonomy requires critical revision (Slavík 2004; Semple and Cook 2006; Kiełtyk and Mirek 2014; Semple et al. 2015; Szymura et al. 2015). As a consequence, it is sometimes hard to decide if ‘intermediate’ morphotypes represent true interspecific hybrids or only the extreme forms of *S. canadensis* or *S. virgaurea*. According to Nilsson (1976), *S. × niederederi* is usually more or less intermediate between *S.*

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canadensis and *S. virgaurea*, especially in traits such as leaf shape and venation and size of capitulum. The hybrid can be also characterized by intermediate size of pollen grains and achenes; however, its pollen viability is reduced as well as achene development (Migdałek et al. 2014; Karpavičienė and Radušienė 2016).

Although interspecific hybridization in the genus *Solidago* is considered to be relatively common (Nesom 1993), proper identification of the parentage for the hybrid individuals solely based on morphological features can be misleading (Schilling et al. 2008). Hybrid nature of *S. × niedereideri* and its parentage has not been independently proven using molecular tools, to date. In this paper, therefore, we aimed to establish the genetic identity of the parental taxa contributing to the hybrid by means of direct sequencing of chosen DNA regions, namely nuclear ribosomal Internal Transcribed Spacer (ITS) and chloroplast intergenic spacer *rpl32-trnL*.

Materials and methods

Plant material and sampling

The study was focused on four taxa of *Solidago* occurring in Europe, including three alien taxa, *S. canadensis*, *S. gigantea* and *S. × niedereideri*, and one native species, *S. virgaurea*. Individuals representing each species were morphologically identified based on diagnostic features given by Nilsson (1976); Slavík (2004); Semple and Cook (2006) and Semple et al. (2015). *Solidago canadensis* was treated in its broad sense (*sensu lato*), including two varieties recognized in its native range (Semple et al. 2015). DNA was extracted from 15 samples of *Solidago*: three samples of *S. × niedereideri*, six samples of *S. canadensis*, five samples of *S. virgaurea* and, as a reference, one sample of *S. gigantea* Aiton. All samples were collected in 2014–2015 from distant populations occurring in different areas of Poland. Details of all studied specimens are summarized in Table 1. In each case one individual per population was sampled. Fresh leaves were collected in the field and stored in silica gel. The voucher specimens of molecularly examined individuals of *S. × niedereideri* were deposited at the Herbarium of the Institute of Botany of the Jagiellonian University in Kraków (KRA 0449366–0449370). To check the potential intraspecific sequence polymorphism, all accessions of the analysed regions for these focal taxa

deposited in GenBank were retrieved and included in the analysis (Table 2).

DNA isolation, PCR amplification and direct sequencing

The amount of 10–15 mg of dried plant material was used for DNA isolation. The plant tissue was ground to fine powder using Mixer Mill 400 (Retsch) and 3 mm tungsten beads. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's protocol. The nuclear ribosomal Internal Transcribed Spacer region (including ITS1, 5.8S and ITS2) and the *rpl32-trnL* intergenic spacer, reported by Shaw et al. (2007) to be highly variable, was amplified in each studied sample. Primers, mix compositions and PCR conditions were as provided by Zalewska-Gałosz et al. (2010). PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics) according to the manufacturer's protocol and sequenced in two directions using the primers used for amplification. Sequencing was performed using BigDye Terminator v.3.1 (Applied Biosystems) with supplied 5× sequencing buffer, according to the manufacturer's manual. Sequencing products were purified using the Ethanol/EDTA protocol, resuspended in 12 µl formamide and separated on an ABI 3100-Avant Genetic Analyser using 50 cm capillaries and POP-6 polymer (Applied Biosystems). Raw sequencing profiles were analysed with the DNA Sequencing Analysis Software v.5.1 (Applied Biosystems). The sequences were manually verified/adjusted using the software Finch TV v.1.4.0 (Geospiza Inc.). Alignments of sequences for all regions were conducted manually using BIOEDIT v.5.0.9. (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Nucleotide polymorphisms were examined with two strands to ensure their consistency and coded using the IUPAC nucleotide ambiguity codes. The GenBank accession numbers for ITS and *rpl32-trnL* sequences obtained in the study are provided in Table 1.

Results

Variation of ITS and analysis of the hybrid individuals

ITS sequences obtained from the studied samples were 691–711 base pair long and their alignment covered

Table 1 Samples of *Solidago* taxa used in the study, their DNA numbers, GenBank accession numbers and geographical origin

Species	DNA sample no.	GB accession no. ITS/ <i>rpl32-trnL</i>	Origin and date of collection	Habitat	GPS coordinates
<i>S. × niedereideri</i>	346	KU872554/ KU872564	Mieruniszki, NE Poland, 20 Aug 2014	Dry roadside ditch	54°10.784'N 22°33.585'E
<i>S. × niedereideri</i>	347	KU872555/ KU872566	Ostrowo near Pluszkiejmy, NE Poland, 7 Sep 2014	Abandoned field	54°17.444'N 22°27.831'E
<i>S. × niedereideri</i>	348	KU872553/ KU872565	Budzów near Jachówka, S Poland, 30 Sep 2014	Abandoned field	49°45.096'N 19°40.636'E
<i>S. canadensis</i>	1102	KU872542/ KU872557	Bibice, S Poland, 24 Jun 2015	Abandoned field	50°06.682'N 19°57.626'E
<i>S. canadensis</i>	1103	KU872544/ KU872556	Białystok, NE Poland, 11 Aug 2015	Ruderal ground	53°07.774'N 23°08.103'E
<i>S. canadensis</i>	1150	KU872543/ KU872570	Gołdap, NE Poland, 30 July 2015	Former railway embankment	54°18.832'N 22°18.827'E
<i>S. canadensis</i>	1152	KU872545/ KU872568	Lubień, S Poland, 8 Sep 2015	Abandoned field	49°43.550'N 19°59.737'E
<i>S. canadensis</i>	1153	KU872546/ KU872569	Warszawa, central Poland, 29 Sep 2015	Ruderal ground	52°13.364'N 20°57.871'E
<i>S. canadensis</i>	1151	KU872547/ KU872567	Jędrzejów, S Poland, 27 Aug 2015	Abandoned field	50°37.770'N 20°16.856'E
<i>S. gigantea</i>	1101	KU872541/ KU872558	Węgrzce near Kraków, S Poland, 24 Jun 2015	Roadside verge	50°06.537'N 19°57.922'E
<i>S. virgaurea</i>	1104	KU872548/ KU872559	Rutka-Tartak, NE Poland, 4 Aug 2015	Roadside slope	54°19.753'N 22°57.583'E
<i>S. virgaurea</i>	1146	KU872549/ KU872560	Filipów Trzeci, NE Poland, 16 Aug 2015	Abandoned field	54°09.543'N 22°37.570'E
<i>S. virgaurea</i>	1147	KU872551/ KU872561	Żelazki, NE Poland, 12 Aug 2015	Abandoned field	54°13.617'N 22°27.534'E
<i>S. virgaurea</i>	1148	KU872552/ KU872562	Połaniec, S Poland, 23 Aug 2015	Abandoned field	50°25.585'N 21°16.022'E
<i>S. virgaurea</i>	1149	KU872550/ KU872563	Alwernia, S Poland, 17 Sep 2015	Abandoned field	50°04.602'N 19°33.379'E

672 bp. Ten polymorphisms were detected in the data set (Table 2). All samples of *Solidago virgaurea* had identical sequences and did not have any intra-individual polymorphism. The ITS sequence of this species was the most divergent and was clearly differentiated from *S. canadensis* and/or *S. gigantea* by eight single nucleotide polymorphism sites (Table 2). Within samples of *S. gigantea* and *S. canadensis* sensu lato intra-individual polymorphism was detected at three positions of the alignment (249, 330 and 509). At position 330, more than half of the studied samples of *S. canadensis*, *S. gigantea* and *S. × niedereideri* showed a sequence ambiguity what suggests the presence of paralogs within the genomes

studied. Incomplete homogenization at this site, however, does not influence the clear inference of the hybrid nature of *S. × niedereideri*, which is possible based on remaining polymorphic sites. Only one, taxonomically specific position (465) allowed to distinguish between *S. gigantea* and *S. canadensis*.

All three accessions of the hybrid *S. × niedereideri* showed heterozygosity at seven positions, all diagnostic and pointed to *S. virgaurea* as one of the parental species. The second parent was detected based on the polymorphism expressed at position 465 at the alignment – the only one position that distinguished *S. canadensis* and *S. gigantea*. At this position, the hybrid samples possessed C while *S.*

Table 2 Polymorphism in the ITS sequences from the *Solidago* taxa and the hybrid individuals of *S. × niedereideri*

Taxon	GB accession no.	Position in the alignment									
		249	330	385	432	465	509	550	606	607	608
<i>S. gigantea</i>	HQ142592, HQ142593, DQ005980, KU872541	T	G	C	C	T	C	G	G	T	G
<i>S. gigantea</i>	HQ142594, HQ142595, EU125362, KP153086	.	R	.	.	.	T
<i>S. canadensis</i>	FJ859719, EU125361, AF477665, KU872544, KU872545, KU872546, KU872551, KU872543	T	R	C	C	C	T	G	G	T	G
<i>S. canadensis</i>	KU872542	.	G
<i>S. canadensis</i> var. <i>scabra</i>	HQ142590	.	G
<i>S. canadensis</i> var. <i>scabra</i>	U97646	C
<i>S. virgaurea</i>	EU125358, KU872548–52	T	G	T	A	C	C	A	T	C	A
<i>S. × niedereideri</i>	KU872553, KU872554	T	R	Y	M	C	Y	R	K	Y	R
<i>S. × niedereideri</i>	KU872555	.	G

Polymorphic nucleotide sites are coded using the IUPAC code

Dots refer to the identical nucleotides provided above for each species

GB accession numbers of the samples collected for the purpose of this study are evidenced in Table 1

gigantea had T, similar to *S. virgaurea* and *S. canadensis*. Considering above, the second parent of the hybrid *S. × niedereideri* was proved to be *S. canadensis*.

Variation of *cpDNA* region and identification of the maternal species

The sequences of the *rpl32-trnL* intergenic spacer were 695–732 base pair long and their alignment counted 732 bp. The data set was only weakly polymorphic, with three polymorphisms detected: one, 30 to 37-bp long insertion/deletion (indel), at position 256–292 and two single nucleotide polymorphisms at positions 177 and 426 of the alignment (Table 3). The long insertion/deletion clearly distinguished *Solidago virgaurea* while the other species, namely *S. canadensis* and *S. gigantea*, cannot be differentiated based on the *rpl32-trnL* sequence. Moreover, *S. canadensis* expressed inter-individual polymorphism in this region. Accessions KU872556, KU872567, KU872569 and KU872570 had C at position 426 of the alignment while accessions KU872557 and KU872568 had T at this position. The *rpl32-trnL* sequence obtained from the studied hybrid individuals was not identical. On the basis of detected polymorphism, it can be stated that the hybrid

individual collected in Mieruniszki (accession KU872564) inherited *cpDNA* from *S. virgaurea*, while the individual collected in Budzów near Jachówka (accession KU872565) had *cpDNA* haplotype alike *S. canadensis* (accessions KU872557 and KU872568). The last hybrid individual collected in Ostrowo near Pluszkiejmy had identical *rpl32-trnL* sequence as *S. canadensis* (accessions KU872556, KU872567, KU872569, KU872570) and *S. gigantea* (accession KU872558). *Solidago gigantea*, however, was excluded from the parentage of *S. × niedereideri* based on ITS polymorphism.

Based on the examination of the *rpl32-trnL* sequence, it can be concluded that hybridization occurs in both directions with maternal inheritance of a specific plastid genome from *S. virgaurea* and *S. canadensis*.

Discussion

In this paper we test the molecular character of *Solidago × niedereideri* from three distant populations occurring in Poland. We show that the hybrid individuals retained both copies of ITS sequences inherited from their parental species, *S. virgaurea* and *S. canadensis*. This finding confirmed the hybrid

Table 3 Sequence variation in the *rpl32-trnL* chloroplast intergenic spacer in the *Solidago* species and the hybrid individuals of *S. × niederederi*

Taxon	GB accession no.	Position in the alignment		
		177	256–292	426
<i>S. gigantea</i>	KU872558	C	–	C
<i>S. canadensis</i>	KU872556, KU872567, KU872569, KU872570	C	–	C
<i>S. canadensis</i>	KU872557, KU872568	C	–	T
<i>S. virgaurea</i>	KU872559	A	ATGTCTAAAAGAATAATTCTTGTATTTTCT	C
<i>S. virgaurea</i>	KU872560–63	C	ATGTCTAAAAGAATAATTCTTGTATTTTCTGAATTCT	C
<i>S. × niederederi</i>	KU872566	C	–	C
<i>S. × niederederi</i>	KU872565	C	–	T
<i>S. × niederederi</i>	KU872564	C	ATGTCTAAAAGAATAATTCTTGTATTTTCTGAATTCT	C

All samples are evidenced in Table 1

origin of *S. × niederederi* as well as its parentage originally proposed by Khek (1905). Although *nrDNA* genes undergo concerted evolution relatively quickly (e.g., Fuertes Aguilar et al. 1999), additive polymorphism pattern detected in the ITS region has been successfully used in the previous studies on inter-specific hybridization in many different genera of plants (e.g., Soltis et al. 1995; Zalewska-Gałosz and Ronikier 2012; Zalewska-Gałosz et al. 2014; Bobrov et al. 2015). A lack of any signs of recombination or homogenization between both parental ITS ribotypes may suggest relatively recent forming of hybrid individuals and/or lack of generative propagation. On the other hand, relatively easy formation of hybrid individuals (both directions of crossing evidenced in this study) and some viability of pollen detected in *S. × niederederi* (Migdałek et al. 2014) could be a prerequisite for possible existence of gene flow between parental species through the hybrid individuals as a genetic ‘bridge’. This can be especially evolutionary important because *S. × niederederi* is an example of spontaneous interspecific hybrid between an invasive and a native species. Such hybridization, together with introgression, competitive exclusion and niche displacement are pointed out as specific pathways in which invasive species may be altering the evolutionary history of native species (Vilà et al. 2000; Guo 2014). The knowledge of hybrid fertility in *S. × niederederi* as well as possible introgression between *S. canadensis* and *S. virgaurea* is still lacking. It cannot be excluded that *S. × niederederi* could form advanced recombinant generations of hybrids

(F₂, F₃...etc.) or backcrosses with the parental species. Because a direct loss of genetic distinctiveness via genetic erosion is possible, areas where *S. virgaurea* is rare or occurs in isolated populations should be protected against the invasion of *S. canadensis*.

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